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Original Research

BRAF V600E status may facilitate decision-making on active surveillance of low-risk papillary thyroid microcarcinoma

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KEYWORDS
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Abstract ***Introduction:*** Conservative active surveillance has been proposed for low-risk papillary thyroid microcarcinoma (PTMC), defined as ≤1.0 cm and lacking clinical aggressive features, but controversy exists with accepting it as not all such PTMCs are uniformly destined for benign prognosis. This study investigated whether *BRAF* V600E status could further risk stratify PTMC, particularly low-risk PTMC, and can thus help with more accurate case selection for conservative management.
Methods: This international multicenter study included 743 patients treated with total thyroidectomy for PTMC (584 women and 159 men), with a median age of 49 years (interquartile range [IQR], 39–59 years) and a median follow-up time of 53 months (IQR, 25–93 months).
Results: On overall analyses of all PTMCs, tumour recurrences were 6.4% (32/502) versus 10.8% (26/241) in *BRAF* mutation-negative versus *BRAF* mutation-positive patients ($P = 0.041$), with a hazard ratio (HR) of 2.44 (95% CI (confidence interval), 1.15–5.20) after multivariate adjustment for confounding clinical factors. On the analyses of low-risk PTMC, recurrences were 1.3% (5/383) versus 4.3% (6/139) in *BRAF* mutation-negative versus *BRAF* mutation-positive patients, with an HR of 6.65 (95% CI, 1.80–24.65) after adjustment for confounding clinical factors. *BRAF* mutation was associated with a significant decline in the Kaplan–Meier recurrence-free survival curve in low-risk PTMC.
Conclusions: *BRAF* V600E differentiates the recurrence risk of PTMC, particularly low-risk PTMC. Given the robust negative predictive value, conservative active surveillance of *BRAF* mutation-negative low-risk PTMC is reasonable whereas the increased recurrence risk and other well-known adverse effects of *BRAF* V600E make the feasibility of long-term conservative surveillance uncertain for *BRAF* mutation-positive PTMC.

1. Introduction

Papillary thyroid cancer (PTC) is a common endocrine malignancy with a rapidly rising incidence globally in recent decades [1–4]. Papillary thyroid microcarcinoma (PTMC), defined as tumour ≤1.0 cm in its greatest dimension [5], constitutes a major portion of thyroid cancer, close to 50% of all thyroid malignancies in some studies [1,6,7]. The Surveillance, Epidemiology, and End Results (SEER) data have shown an increase in the incidence of PTMC from 3.39 per 100,000 person-years in 1983–1985 to 13.02 per 100,000 person-years in 2010–2013, with an average annual percent change of 9.3% [1]. Although the majority of patients with PTMC have an indolent disease course with no serious clinical outcomes, some patients do have tumour metastasis and even disease-specific mortality [8–10]. Of particular note is that recurrence of PTMC can occur after treatment, which may be associated with increased risk of patient morbidity and mortality. Thus, prevention of disease recurrence of PTMC is a major goal of its treatment in the current clinical practice.

It is often difficult to precisely risk stratify and accordingly treat PTMC to achieve an optimal benefit-harm balance of the treatments. Therefore, the management style of PTMC, particularly low-risk PTMC, is currently widely variable in different clinical practices around the world, ranging from total thyroidectomy for every patient with PTMC to non-surgical active surveillance in patients with clinically low-risk PTMC. The conservative active surveillance is attractive but controversial as an approach to clinically low-risk PTMC, defined as lack of extrathyroidal extension (ETE), lymph node metastasis (LNM) and distant metastasis (DM), which are well known to be associated with an increased risk of recurrence [9–11]. One major issue is that in some patients initially apparently low-risk PTMC is actually inherently destined, perhaps genetically driven, for a later course of poor prognosis—disease recurrence and even mortality. To identify those patients with PTMC that has clinically apparently low-risk at the initial presentation but has inherently high potential for poor prognosis, such as disease recurrence, is clinically challenging. The opposite is also true—i.e., it may not be always straightforward to identify the cases with apparently low-risk PTMC that is truly of low risk. A novel approach, such as the use of genetic guidance, which could further risk stratify clinically apparently low-risk PTMC, could be useful in helping more precisely to define the management of this common type of thyroid cancer.

In recent years, *BRAF* V600E mutation has been introduced as a genetic prognostic marker to assist the risk evaluation of PTC [12,13]. There have also been some studies on PTMC in this regard, but they have been virtually all focused on the overall analyses of

PTMC with inconsistent results [11,14,17]. There are limited data on the prognostic value of *BRAF* V600E in the unique category of PTC—clinically low-risk PTMC. More than ten years ago, Mazzaferri suggested that *BRAF* V600E could be useful in further risk stratification of low-risk PTMC [18], but current clinical guidelines on the treatment of thyroid cancer, such as the American Thyroid Association guidelines, have not been able to specifically define yet *BRAF* V600E as a risk-differentiating factor for low-risk PTMC [19]. This controversy derives largely from the fact that direct data on the prognostic value of *BRAF* V600E in the risk assessment of low-risk PTMC is lacking. There has been particularly lack of a large multicenter study that could provide a strong analysis power to resolve this controversy. In view of this, we conducted the present large international multicenter study to directly investigate the prognostic value of *BRAF* V600E mutation in PTMC, with a particular emphasis on low-risk PTMC.

2. Materials and methods

2.1. Patients and mutational analyses

This study initially included 2638 patients with PTC from 11 medical centres in six countries, as detailed previously [20–24]. After exclusion of patients with tumour size >1.0 cm, we had 743 patients with PTMC (584 women and 159 men), with a median age of 49 years (interquartile range [IQR], 39–59 years) and a median clinical follow-up time of 53 months (IQR, 25–93 months) from 1978 to 2015. The clinicopathological demographic characteristics of the patients with PTMC in this cohort from different medical centres are presented in Supplemental Table S1. We defined low-risk PTMC as having no ETE, LNM, and DM and high-risk PTMC as having at least one of these high-risk characteristics. All patients received total or near-total thyroidectomy. Neck lymph node dissection and radioiodine ablation were pursued when clinically indicated as previously described [16,22,23]. Tumour recurrence was defined as combined persistent and recurrent disease confirmed by histologic/cytologic/radiographic/biochemical criteria as previously described [16]. Follow-up time was defined as the time from initial thyroidectomy to tumour recurrence or to the latest clinical contact in the case of no recurrence. The study was approved by the institutional review board of all the centres involved and informed consent was obtained from patients where required. For *BRAF* V600E mutation analyses, we amplified exon 15 of the *BRAF* gene containing the mutation hotspot using polymerase chain reaction primers, as described previously [24–35]. Genetic analyses were performed after surgical and radioiodine ablation treatments in all patients and the *BRAF* mutation status did not affect the treatment strategy.

2.2. Statistical analysis

We presented continuous data as medians and IQRs using the Wilcoxon–Mann–Whitney test for the analysis of non-normally distributed variables and presented categorical data as frequencies and percentage using a chi-squared test for the analysis or Fisher's exact test for small case numbers. Kaplan–Meier survival curves with log-rank test were used to analyse the recurrence-free survival. Independent risk factors associated with disease recurrence were examined by Cox-regression analyses to generate hazard ratio (HR) and 95% confidence interval (CI). All reported *P* values were two-sided and a value <0.05 was considered significant. All analyses were performed using SPSS version 20.0 (IBM SPSS, Inc. New York, NY) and GraphPad Prism version 7 (GraphPad Software, San Diego, CA).

3. Results

3.1. Effect of BRAF V600E mutation on disease recurrence of PTMC in the overall cohort

We first took the advantage of this large multicenter cohort of 743 patients with PTMC to examine the general effect of *BRAF* V600E mutation on tumour behaviours, particularly disease recurrence, in the overall PTMC cohort (Table 1). The overall prevalence of *BRAF* V600E mutation in PTMC was 32.4% (241/743). *BRAF* V600E was associated with several high-risk tumour behaviours, such as ETE and LNM. The tumor recurrence rate in patients with *BRAF* mutation-negative PTMC was significantly lower compared with that of *BRAF* mutation-positive patients (6.4% versus 10.8%, respectively), with an unadjusted HR of 2.01 (95% CI, 1.20–3.38), which remained significant at 2.44 (95% CI, 1.15–5.20) after adjustment for patient age, sex, conventional pathological risk factors, medical centre, and radioactive iodine treatment (Table 2). A significant association between *BRAF* V600E mutation and decreased recurrence-free survival is also revealed on Kaplan–Meier analysis (log-rank $P = 0.007$; Fig. 1A).

Similar results were obtained when the analyses were performed only on a conventional variant of PTMC (CPTMC). The prevalence of *BRAF* mutation in this group was 32.9% (198/602). Tumour recurrence rates in CPTMC were 6.2% (25/404) versus 12.1% (24/198) in *BRAF* mutation-negative and *BRAF mutation*-positive patients, respectively, with an HR of 2.94 (95% CI, 1.20–7.20) after adjustment for patient age, sex, conventional pathological risk factors, medical centre, and radioactive iodine treatment (Table 2). On Kaplan–Meier analysis, the recurrence-free survival curve of *BRAF* mutation-positive patients significantly

Table 1
Clinicopathological demographic characteristics of papillary thyroid microcarcinoma in patients according to the *BRAF* genotype.

	All patients	*BRAF* V600E negative	*BRAF* V600E positive	*P*-value
Total case, n (%)	743	502 (67.6)	241 (32.4)	
Age, median (interquartile range (IQR))	49 (39–59)	48 (39–59)	49 (39–59)	0.706
Female, n (%)	584 (78.6)	392 (78.1)	192 (79.7)	0.623
Tumour size, median (IQR), cm	0.8 (0.5–1.0)	0.7 (0.5–1.0)	0.8 (0.6–1.0)	<0.001
Extrathyroidal extension, n (%)	98 (13.2)	44 (8.8)	54 (22.4)	<0.001
Lymph node metastasis, n (%)	168 (22.6)	95 (18.9)	73 (30.3)	0.001
Vascular invasion, n (%)	16/319 (5.0)	11/257 (4.3)	5/62 (8.1)	0.209
Multifocality, n (%)	281 (37.8)	172 (34.3)	109 (45.2)	0.004
Distant metastasis, n (%)	11 (1.5)	7 (1.4)	4 (1.7)	0.754
Stage (AJCC 8th), n (%), (n = 732)				
I	691 (93)	474 (95.4)	217 (92.3)	0.173
II	38 (5.1)	21 (4.2)	17 (7.2)	
III	0	0	0	
IV	3 (0.4)	2 (0.4)	1 (0.4)	
^{131}I treatment, n (%)	447/742 (60.2)	257/501 (51.3)	190/241 (78.8)	<0.001
^{131}I dose, median (IQR), mCi	50 (0–100)	30 (0–100)	100 (30–101)	<0.001
Follow time, median (IQR), months	53 (25–93)	56 (29.5–99)	40 (21–83)	<0.001
Tumour recurrence, n (%)	58 (7.8)	32 (6.4)	26 (10.8)	0.041

Table 2
Hazard ratios of *BRAF* V600E mutation for recurrence of PTMC on the overall analysis.

	Recurrence n (%)	Unadjusted		Adjusted[a]	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
All PTMCs					
BRAF V600E negative	32/502 (6.4)	1 (reference)		1 (reference)	
BRAF V600E positive	26/241 (10.8)	2.01 (1.20–3.38)	0.008	2.44 (1.15–5.20)	0.020
Conventional PTMC					
BRAF V600E negative	25/404 (6.2)	1 (reference)		1 (reference)	
BRAF V600E positive	24/198 (12.1)	2.29 (1.30–4.01)	0.004	2.94 (1.20–7.20)	0.018

PTMC, papillary thyroid microcarcinoma; CI, confidential interval.
[a] Adjusted for patient age at diagnosis, sex, tumour size, cervical lymph node metastasis, extrathyroidal extension, vascular invasion, multifocality, medical centres, and radioactive iodine treatments.

decreased compared with that of *BRAF* mutation-negative patients (log-rank $P = 0.003$; Fig. 1B).

3.2. Effect of BRAF V600E mutation on disease recurrence of low- and high-risk PTMCs

We next examined the effect of *BRAF* V600E mutation on disease recurrence of PTMC in different risk groups. As shown in Table 3, the overall *BRAF* V600E rate was 26.6% (139/522) and disease recurrence rate was 2.1% (11/522) in low-risk PTMC. The recurrence rates were 1.3% versus 4.3% in *BRAF* mutation-negative versus *BRAF mutation*-positive patients, respectively, with a HR of 6.65 (95% CI, 1.80–24.65) after adjustment for patient age, sex, medical centre, and radioactive iodine treatment. The negative predictive value of *BRAF* mutation for recurrence of low-risk PTMC was 98.7% (95% CI, 96.8%–99.5%) (Table 3). In the high-risk group, however, *BRAF* mutation had no significant effect on tumour recurrence, with a HR of 1.28 (95% CI, 0.69–2.37) after adjustment for patient age, sex, medical centre, and radioactive iodine treatment. On Kaplan–Meier analysis, *BRAF* mutation was associated with a significant decrease in recurrence-free survival curve in low-risk PTMC (log-rank $P = 0.023$; Fig. 2A), whereas in the high-risk group, *BRAF* mutation had no significant effect on the recurrence-free survival curve (log-rank $P = 0.688$; Fig. 2B).

Similar results were obtained in CPTMC. The recurrence rate in low-risk CPTMC was 1.3% versus 4.3% in *BRAF* mutation-negative versus *BRAF mutation*-positive patients, with a HR of 5.15 (95% CI, 1.21 to 21.83) after adjustment for patients age, sex, medical centre, and radioactive iodine treatment (Table 3). In high-risk CPTMC, *BRAF* mutation had no effect on tumour recurrence, with an adjusted HR of 1.34 (95% CI, 0.71 to 2.56). On Kaplan–Meier analyses, *BRAF* mutation was associated with a significant decline in the recurrence-free survival curve in low-risk CPTMC (log-rank $P = 0.036$; Fig. 2C), but not in high-risk CPTMC (log-rank $P = 0.422$; Fig. 2D).

4. Discussion

It can be a challenging task to precisely risk stratify patients with PTMC for prognostic risk level-based

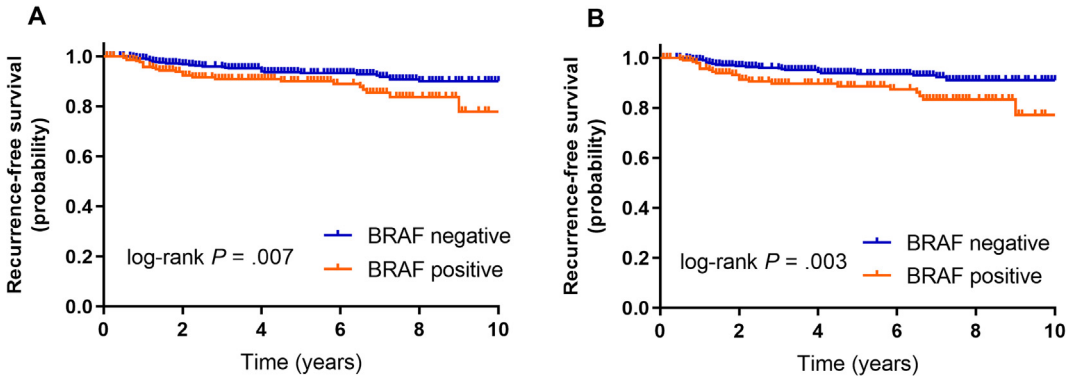

Fig. 1. Kaplan–Meier analysis of disease recurrence-free survival by *BRAF* V600E mutation status in papillary thyroid microcarcinoma (PTMC). A. All types of PTMC. B. Conventional variant of PTMC. Comparison of recurrence-free survival was performed between *BRAF* V600E mutation-positive and wild-type *BRAF* patients using the log-rank test. Follow-up time is truncated at 10 years.

appropriate managements, particularly apparently low-risk cases. Although patients with PTMC generally have an excellent prognosis [36,37], some patients experience tumour recurrence and even mortality [9,10], suggesting that all PTMCs do not have the same intrinsic risk for poor outcomes. There are currently ongoing debates particularly on how to further risk stratify and appropriately manage clinically apparently low-risk PTMC. Controversies exist particularly on how to select cases of such low-risk PTMC for conservative active surveillance.

In this context, the present large international multicenter study has demonstrated that *BRAF* V600E mutation can be a prognostic marker for poorer clinicopathological outcomes of PTMC, particularly for disease recurrence of low-risk PTMC. In fact, this study has for the first time shown that *BRAF* mutation can further differentiate the recurrence risk of clinically low-risk PTMC—wild-type *BRAF* patients have an extremely low risk of recurrence whereas *BRAF* mutation patients have a significantly increased recurrence risk, representing an independent prognostic value of *BRAF* mutation in the apparently clinically low-risk PTMC. Unlike in low-risk PTMC, in high-risk PTMC, *BRAF* V600E mutation was not an independent risk factor for disease recurrence in the present study. This finding may be expected, given the fact that even in the absence of *BRAF* mutation, classical high-risk tumour features already existed in high-risk PTMC as defined in the present study, which would be associated with a high recurrence rate. These findings in the present study by analysing low- and high-risk PTMCs separately may now reconcile the inconsistent results of previous studies on the prognostic value of *BRAF* V600E in the overall analyses of all PTMCs as the outcomes of those studies would vary depending on the compositae portions of low- and high-risk PTMCs in the cohorts of patients included [11,14–17].

Active surveillance has been recently proposed as an alternative option to surgical treatment in low-risk PTMC, which is drawing increasing attention [19,38,39]. It is of concern, however, that all low-risk PTMCs may not uniformly remain "silent" without clinical consequences [40–42]. It is also unknown what molecular markers can distinguish intrinsically aggressive but initially apparently low-risk PTMC from truly indolent low-risk PTMC. A striking finding in the present study was the extremely low recurrence rate in low-risk PTMC that harboured the wild-type *BRAF*, representing a robust negative predictive value (99%) of *BRAF* V600E for disease recurrence. It has been recently recommended by Miyauchi and Ito that active surveillance is the primary approach to the management of clinically low-risk PTMC as opposed to immediate surgical treatment [43]. Our present study suggests that this conservative approach is reasonable for *BRAF* mutation-negative low-risk PTMC given its extremely low recurrence rate. This is supported also by the fact that virtually no PTC-related mortality occurred in patients with *BRAF* mutation-negative PTC, particularly in patients with conventional PTC, including even PTC >1.0 cm [44]. In this context, because the cost of *BRAF* test is generally low and the cost of thyroidectomy is high, this *BRAF* status-based approach to the management of PTMC would spare many patients from total thyroidectomy or even any thyroidectomy and would thus likely be cost-saving, in addition to other advantages.

Our present study demonstrated that *BRAF* V600E mutation could independently define a significantly increased risk of disease recurrence in initially apparently low-risk PTMC. This finding, together with the well-known other adverse effects of *BRAF* V600E on PTC [12,13,16,24], suggests that non-surgical long-term surveillance may not be appropriate for patients with *BRAF* mutation-positive low-risk PTMC. Even a recurrence rate of 4.3% in the *BRAF* mutation-positive low-risk PTMC found in the present study seems to be relatively low; this significant increase in recurrence risk compared with *BRAF* mutation-negative PTMC suggests a significantly increased aggressive potential of the tumour associated with *BRAF* V600E. This is a concern

Table 3
Hazard ratios of *BRAF* V600E mutation for recurrence of PTMC in low- and high-risk groups.

	tumour recurrence				Unadjusted		Adjusted[a]		Negative predictive value, % (95% CI)
	Overall, n (%)	*BRAF* V600E negative, n (%)	*BRAF* V600E positive, n (%)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	
All PTMCs									
Low-risk group	11/522 (2.1)	5/383 (1.3)	6/139 (4.3)	0.076	3.63 (1.11–11.91)	0.033	6.65 (1.80–24.65)	0.005	98.7 (96.8–99.5)
High-risk group	47/221 (21.3)	27/119 (22.7)	20/102 (19.6)	0.577	1.13 (0.63–2.02)	0.689	1.28 (0.69–2.37)	0.437	77.3 (68.5–84.3)
Conventional PTMC									
Low-risk group	9/423 (2.1)	4/308 (1.3)	5/115 (4.3)	0.066	3.71 (1.00–13.85)	0.051	5.15 (1.21–21.83)	0.026	98.7 (96.5–99.6)
High-risk group	40/179 (22.3)	21/96 (21.9)	19/83 (22.9)	0.871	1.29 (0.69–2.41)	0.424	1.34 (0.71–2.56)	0.369	78.1 (68.3–85.7)

PTMC, papillary thyroid microcarcinoma; CI, confidential interval.
[a] Adjusted for patient age at diagnosis, sex, medical centres, and radioactive iodine treatments.

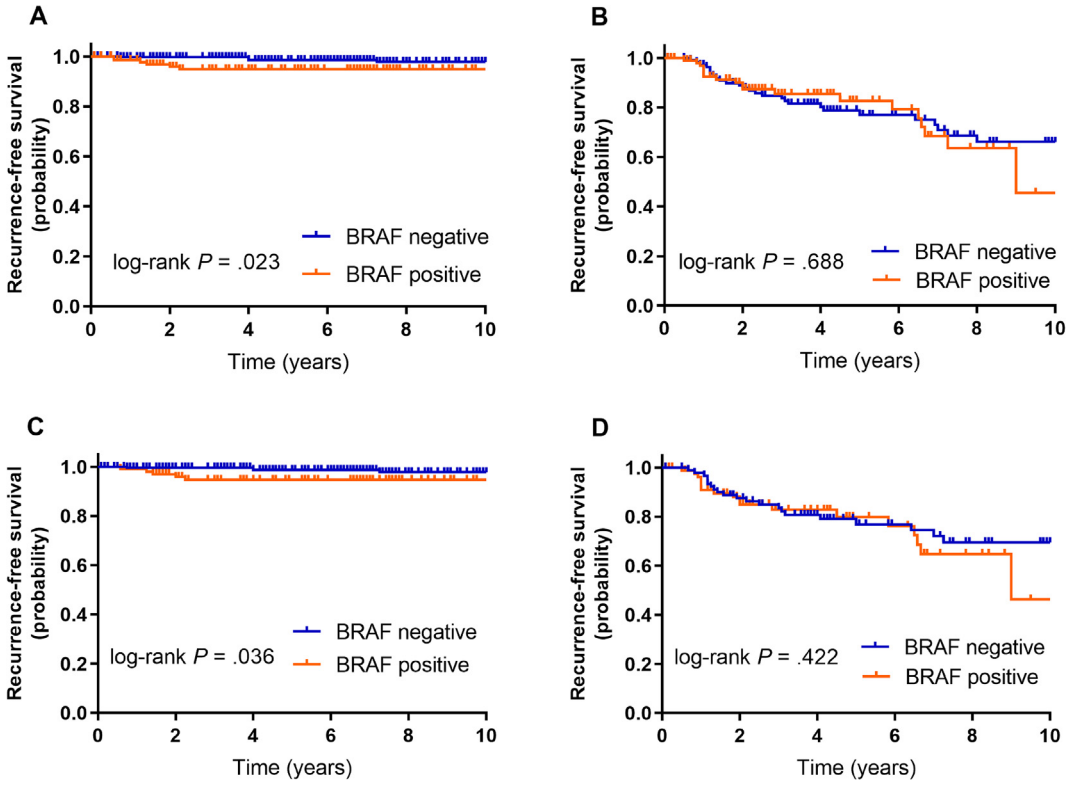

Fig. 2. Kaplan–Meier analysis of disease recurrence-free survival by *BRAF* V600E mutation status in PTMC of different risks. A. Low-risk PTMC; B. high-risk PTMC; C. low-risk conventional PTMC; and D. high-risk conventional PTMC. Comparison of recurrence-free survival was performed between *BRAF* V600E mutation-positive and wild-type *BRAF* patients using the log-rank test. Follow-up time was truncated at 10 years.

particularly given that the long-term impact of *BRAF* mutation on clinical outcomes of thyroid cancer will likely be substantial if significant tumour growth, ETE, or LNM occurs. This is because previous findings suggest that *BRAF* V600E-positive intrathyroidal PTC >1.0 cm has a substantially increased recurrence risk, particularly in the case of tumours >2.0 cm where there was a robustly increased recurrence risk to around 20–30%, which was comparable with the recurrence risk of invasive PTC [21]. It has been previously demonstrated that *BRAF* V600E and ETE or LNM has a robustly synergistic adverse effect on clinical outcomes of PTC, including disease recurrence [16] and patient mortality [24]. New ETE and LNM can develop even in initially low-risk PTMC if given sufficient time and even in the absence of significant growth of the primary tumour. Once ETE and LNM occur in *BRAF* V600E-positive tumour, synergistic interactive effects between the newly developed aggressive pathological factors and *BRAF* V600E on poor clinical outcomes of PTMC may

intensify [16,24]. Moreover, the adverse effects of *BRAF* V600E on clinical outcomes of PTC, such as disease-specific mortality, start to be significantly manifested particularly after 10 years of clinical follow-up from the initial treatment [24]. Therefore, *BRAF* mutation-positive low-risk PTMC has an aggressive potential if left untreated, making uncertain the feasibility of the long-term non-surgical conservative surveillance for such *BRAF* mutation-positive thyroid cancer. It is worth noting that *BRAF* mutation was found only in 26.6% of patients with clinically low-risk PTMC in the present study. Thus, if the *BRAF* mutation status is used to assist the management of low-risk PTMC, the vast majority of patients could be managed with conservative surveillance although only a minority of patients need to pursue thyroidectomy. It has been recently recommended that a clinical risk level-based approach in the prognostic use of *BRAF* mutation to the management of PTC be applied and, as such, thyroid lobectomy may be just adequate for *BRAF* mutation-positive low-risk PTMC [45].

A limitation of the present study was the relatively small number of patients in the high-risk group of PTMC, reducing the power to conclude the results. Previous studies demonstrated that even though *BRAF* mutation-negative PTC could have recurrence [16,46], PTC-related mortality virtually only occurred in patients with *BRAF* mutation-positive PTC [44]. Thus, the *BRAF* mutation status may also have a prognostic value even in high-risk PTMC: absence of the mutation implies virtually no PTMC-related mortality. The lack of information on other mutations, such as rat sarcoma (*RAS*) mutations and telomerase reverse transcriptase (*TERT*) promoter mutation, is another limitation of this study. However, *RAS* mutations are mutually exclusive with *BRAF* V600E [47] and they alone do not have adverse effects on the outcomes of low-risk PTC [45]. *TERT* promoter mutation is uncommon in PTMC, which alone is also not associated with aggressiveness of PTMC [48]. We analysed the Johns Hopkins cases, which had information on both the *BRAF* and *TERT* mutations, and found that *TERT* promoter mutation alone indeed had no effect, whereas *BRAF* mutation alone had a significant effect on disease recurrence either in the overall analysis of all PTMCs or low-risk PTMC; in fact, a remarkable recurrence rate of 13.5% (5/37) was observed in the group with *BRAF* mutation alone versus only 1.6% (3/189) in the group with no mutation (Supplemental Table S2). *BRAF* V600E and *TERT* promoter mutations often coexist in PTC to form an oncogenic genetic duet that is associated with a robustly increased risk of poor clinical outcomes of PTC [44,46]. The number of cases with this genetic duet was too small in PTMC, particularly low-risk PTMC, to analyse in the present study (Supplemental Table S2).

In summary, in this large multicenter study, we demonstrate that *BRAF* V600E can further differentiate the prognostic risk of low-risk PTMC: the mutation has an extremely robust negative predictive value for disease recurrence and is associated with a significantly increased recurrence. These results, together with the known aggressive role of *BRAF* V600E in PTC in general, suggest that *BRAF* mutation-positive PTMC can be reasonably treated surgically; the feasibility of long-term conservative surveillance of *BRAF* mutation-positive PTMC initially presenting with low-risk clinical features seems uncertain, making it reasonable at this time to treat such thyroid cancer surgically, albeit with limited surgical extent—thyroid lobectomy, for example. In contrast, conservative management in the form of non-surgical active surveillance is reasonable for *BRAF* mutation-negative low-risk PTMC, which accounts for the majority of patients with clinically low-risk PTMC. Thus, more precise management of patients with low-risk PTMC can be achieved by including the *BRAF* V600E mutation status in the prognostic risk stratification.

Conflict of interest statement

Mingzhao Xing receives royalties as co-holder of a licensed USA patent related to *BRAF* V600E mutation in thyroid cancer. Other authors have no conflict of interest to disclose.

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Disclaimer

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.10.017.

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